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Dosage of Metankos on Mortality of *Oryctes rhinoceros* L. and Growth of Oil Palm Seedlings in Peaty Area

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ABSTRACT

The primary challenge associated with oil palm cultivation in Riau is the infestation of rhinoceros beetles (*Oryctes rhinoceros*). Research has been conducted on the potential of the local biopesticide *Metarhizium anisopliae* to manage *Oryctes rhinoceros*, specifically through its application in a compost medium called Metankos. The effectiveness of this biopesticide is indicated by its pathogenicity towards *Oryctes rhinoceros* larvae on oil palm seedlings cultivated in peat soil. This study aimed to determine the optimal application dosage of Metankos compost for enhancing *Oryctes rhinoceros* mortality and promoting the growth of oil palm seedlings in peat soil. The experimental design was randomized entirely, consisting of five treatments with five replications. The treatments included varying doses of Metankos compost applied to oil palm nurseries: 0 kg, 0.1 kg, 0.2 kg, 0.3 kg, and 0.4 kg per plant. Observations were conducted to assess several parameters, including the initial time of death, lethal time (LT50), daily mortality rates, total mortality of larvae, plant height, number of fronds, frond length, stem diameter, wet weight, dry weight, temperature, and humidity. The findings of the study indicated that the most effective dosage for inducing mortality in *Oryctes rhinoceros* larvae was 0.4 kg of Metankos per plant, resulting in a 40% mortality rate, an average time to initial death of 3.75 days, and a lethal time (LT50) of 7.8 days; however, this dosage did not achieve the threshold for effectiveness. Conversely, the optimal dosage for promoting the growth of oil palm seedlings, as measured by height, number of fronds, and stem diameter, was also determined to be 0.4 kg per plant.

Keywords: Dose, *Metarhizium anisopliae*, Methancos, *Oryctes rhinoceros*, Seedlings

1. INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is a plantation crop with a high economic value. Oil palm plays an important role in increasing the country's foreign exchange, with the production of palm kernel oil representing a significant contributor to this (Syukri et al., 2019).

The *Oryctes rhinoceros* beetle represents a significant pest species that has a detrimental impact on oil palm plants. The *Oryctes rhinoceros* beetle represents the primary focus of oil palm pest management efforts in Riau Province. The extent of the *Oryctes rhinoceros* infestation in Riau Province has reached 12,384.85 ha, with the most extensive outbreak occurring in Indragiri Hilir Regency (2,717 ha), Siak (340 ha), Kampar (579 ha), and Kuansing (459 ha). The remaining infested areas are distributed across oil palm plantations owned by the local community (Badan Pusat Statistik, 2016).

The population dynamics of *Oryctes rhinoceros* are significantly affected by plentiful food sources, particularly empty oil palm bunches (OPEFB) that are strategically placed on plant plates to enhance nutrient availability. This practice has been shown to promote an increase in the *Oryctes rhinoceros* population. OPEFB is a valuable organic material that improves soil structure and acts as a potent nutrient source (Sakiah et al., 2018). Due to its slow degradation rate, OPEFB requires composting to expedite nutrient availability for plant uptake. Composting transforms solid waste into high-quality organic compost fertilizer (Warsito et al., 2016). By converting nutrient-rich OPEFB waste into compost, the nutrients become readily accessible to plants. Furthermore, the composting of OPEFB has been found to mitigate the incidence of *Oryctes rhinoceros* infestations (Yustina et al., 2011; Fauzana et al., 2023). In efforts to manage *Oryctes rhinoceros* populations, the application of *Metarhizium anisopliae* compost is employed, which also serves as a biopesticide against various pests (Fauzana & Fadilla, 2022).

The control of *Oryctes rhinoceros* relies primarily on utilizing biological agents, namely *Metarhizium anisopliae* (Metsch.). The entomopathogenic fungus *Metarhizium anisopliae* has been investigated in compost media, specifically in the form of methane. The study's findings (Fauzana et al., 2023) indicated that sawdust is the most effective organic material in compost, followed by TKKS. The application of *Metarhizium anisopliae* at a concentration of 75 g.l-1 in methankos resulted in the mortality of 72.5% of *Oryctes rhinoceros* larvae. The advantage of sawdust is that it decomposes rapidly, facilitating composting. However, it should be noted that sawdust contains a lower concentration of nutrients than TKKS. Combining TKKS, which has a higher nutrient content, is highly beneficial with sawdust.

The fungus *Metarhizium anisopliae* produces the toxin destruxin, which is toxic to insects, particularly *Oryctes rhinoceros* (Yuningsih & Widyaningrum, 2014). Destruxin can affect the organelles of target cells, including the mitochondria, endoplasmic reticulum, and nuclear membrane. This results in the cessation of cellular activity and the malfunctioning of vital organs, namely the midgut, malpighian tubules, hemocytes, and muscle tissue. Ultimately, this leads to the insect's demise (Tampubolon et al., 2013).

The use of Metankos from TKKS and sawdust with a concentration of *Metarhizium anisopliae* 50g.l-1 tends to be the best in causing mortality of *Oryctes rhinoceros* larvae of 56% (Fauzana et al., 2020). Furthermore, an increase in the concentration of *Metarhizium anisopliae* 75 g/l (42.8 x 106 conidia) and 80 g/l (45.6 x 106 conidia) was studied, total mortality reached 72.5% and 80%, so it can be categorized as a biopesticide (Fauzana & Fadilla, 2022). TKKS and sawdust compost given

Beauveria bassiana caused mortality of *O. rhinoceros* larvae by 90% (Novianti et al. 2021). The best dose of a liquid formulation of *Metarhizium anisopliae* mixed into the compost was 180 g/l. 5kg-1 compost causes the mortality of *Oryctes rhinoceros* L. larvae to reach 90% (Fauzana et al., 2023).

The application of 30 kg/tree of TKKS compost has been demonstrated to enhance several growth parameters in TBM oil palm, including plant height, number of fronds, chlorophyll content, frond length, and leaf area (Mahyuddin et al., 2019).

Metarhizium anisopliae in compost can potentially regulate *Oryctes rhinoceros* infestation in vitro; however, it is imperative to ascertain the optimal application dose in a field setting on peat soil. The decomposition process in peat soil is not yet fully developed, given its high organic matter content (Nurida et al., 2011). This allows *Oryctes rhinoceros* larvae to thrive in peat. This study aimed to identify the optimal application rate of Metankos compost for both the mortality of *Oryctes rhinoceros* and the growth of oil palm seedlings on peat soil.

2. MATERIAL AND METHOD

The research was conducted at PT. TH Indo Plantation KM 14.5 Simpang Kanan in Pelangiran District, Indragiri Hilir Regency (Figure 1). The research location was on peat soil, April to October 2024.

Figure 1. Geographic coordinate map of the location of PT TH Indo Plantation Indragiri Hilir.

The study used a completely randomized design with 5 treatments, namely the use of Metankos compost doses in oil palm nurseries: Metankos dose 0 kg / plant, 0.1 kg / plant, 0.2 kg / plant, 0.3 kg / plant, Metankos dose 0.4 kg / plant. Each treatment was repeated 5 times, so that there were 25 plants. The results of the variance analysis had a significant effect and were further tested at the DNMRT level of 5%.

2.1. Making Compost

Compost is made from a mixture of organic materials, shredded TKKS, sawdust and manure on a plastic tarpaulin using a ratio of 3:3:2. To accelerate the decomposition of compost, 1200 ml of EM4, 5 g of TSP, 5 g of urea, 5 g of dolomite, 2500 g of brown sugar and water are added until the mixture becomes moist. The materials are made into 4 layers so that the materials are evenly mixed. All materials are watered, stirred and covered using a plastic tarpaulin. The compost is turned over once a week for three months. The characteristics of mature compost are that it is blackish brown, loose, does not have a pungent odor, and the compost media is close to room temperature.

2.2. Reisolation of *Metarhizium anisopliae*

The fungus *Metarhizium anisopliae* was obtained from an isolate from an oil palm. The isolate was re-isolated using PDA media. The isolate of *Metarhizium anisopliae* on PDA media was taken with an ose needle and placed on PDA media in the LAFC room. The petri dish was tightly covered using plastic wrap and incubated for five days..

2.3. Propagation of *Metarhizium anisopliae*

The cracked corn media was washed and boiled until one third cooked. The cracked corn was put into a 0.5 kg plastic bag, stapled, and sterilized in an autoclave. The *Metarhizium anisopliae* fungus in the PDA media was taken with a cork borer and inserted into the cracked corn media in the LAFC room, the media was shaken, and stapled.

2.4. Preparation of *Metarhizium anisopliae* suspension

The best dose of liquid formulation of *Metarhizium anisopliae* mixed into compost is 180 g.l-1.5kg-1 compost (Fauzana et al., 2023). 180 g of *Metarhizium anisopliae* starter is mixed with 1 liter of water in a 1000 ml Erlenmeyer flask, in a rotary shaker for 24 hours and 1 g of granulated sugar is added.

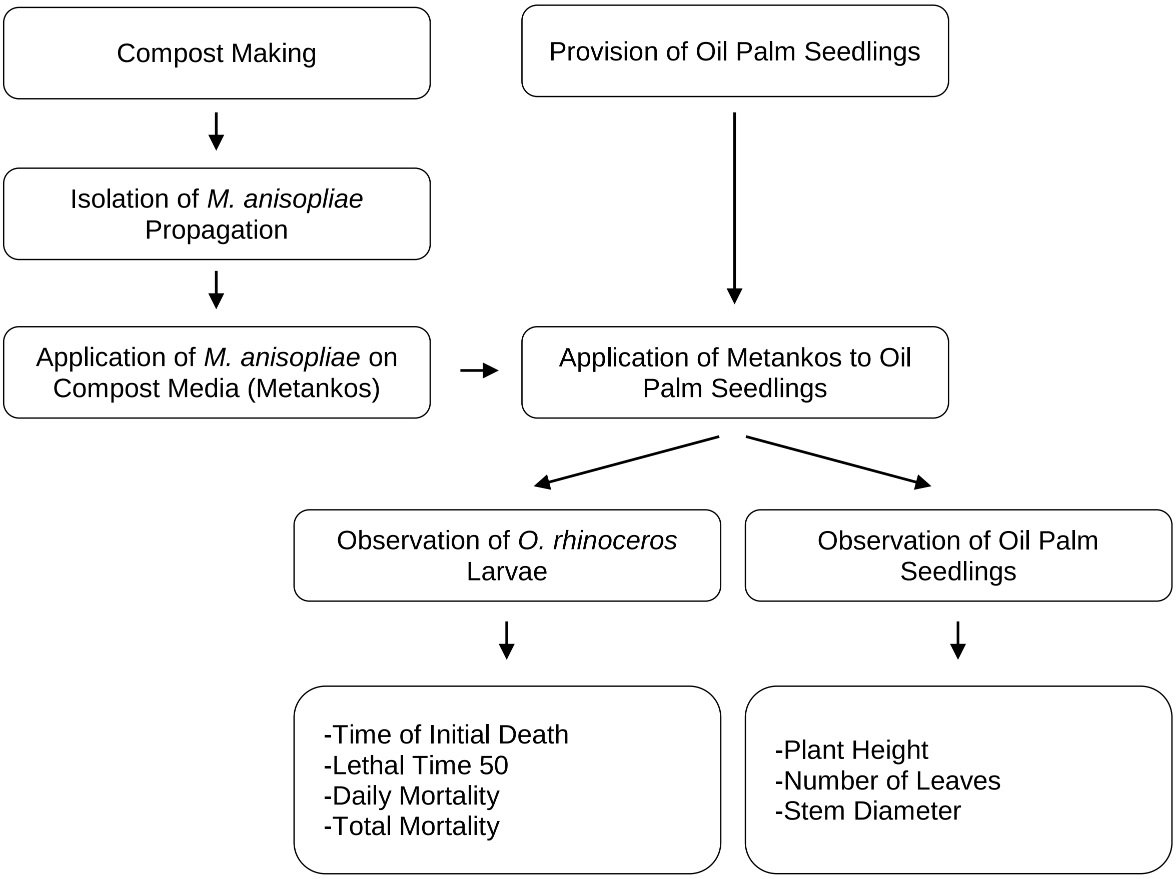

Figure 2. Research Flow Diagram

2.5. Application of *Metarhizium anisopliae* suspension in compost

The mature compost is placed on a tarpaulin and sprinkled with *Metarhizium anisopliae* suspension using a watering can. The mixture is mixed evenly using a hoe. The volume of watering is 500 ml for 5 kg of compost. The methankos media is incubated for 8 days before being applied..

2.6. *Oryctes rhinoceros* larvae infestation and treatment application

Oryctes rhinoceros instar 2 larvae were infested in seed plates, each with

10 larvae. Metankos was sprinkled on seed plates in polybags..

2.7. Observation

Observations include the initial time of death, lethal time (LT50), daily mortality, total mortality, plant height, number of fronds, stem diameter, temperature and humidity of Metankos. The implementation of the research activities is described in the flow diagram presented in Figure 2.

2.8. Data Analysis

The data from the variance analysis showed a real effect, which was further tested using Duncan's New Multiple Range Test (DNMRT) at the 5% level.

3. RESULT AND DISCUSSION

3.1. Early Time of Death

The administration of methancos dose gave a significant difference to the initial time of death with a range of 3.75-6.75 days after application. The dose of methancos 0.4 kg/plant was significantly different from the dose of 0 kg/plant (25 days) but was not significantly different from other treatments, namely doses of 0.3 kg/plant (4.75 days), 0.2 kg/plant (5 days) and 0.1 kg/plant (6.75 days) (Table 1). This is thought to be because administering the dose of methancos containing M. anisopliae conidia that enter the insect's body form few hyphae and require a longer time to kill the *Oryctes rhinoceros* pest. This was stated by (Heriyanto and Suharno, 2008) that the success of using entomopathogenic fungi in pest control is determined by the density and germination power of conidia, the higher the density and germination power, the faster the chance of M. anisopliae killing.

Table 1. Early mortality of *Oryctes rhinoceros* with Metankos application in oil palm nurseries

Metankos dosage	Initial time of death (Days)
0.4 kg/plant	3.75 ± 1.89 a
0.3 kg/ plant	4.75 ± 1.89 a
0.2 kg/ plant	5.00 ± 2.16 a
0.1 kg/ plant	6.75 ± 0.5 a
0 kg/ plant	25.00 ± 0 b

Numbers followed by different lowercase letters are significantly different according to the DNMRT test at the 5% level. After being transformed with √y

Further test results showed no significant difference between treatments (Table 1) because the M. anisopliae conidia were still in the infection process which required time to kill the initial death of *O. rhinoceros* so that it showed the initial death tended to be the same. Environmental factors also affect the initial death, Based on the results of observations in the field, the temperature and humidity in oil palm nurseries were quite high, namely 28.70C while the humidity was quite low, namely 67.98%. This condition is a condition that is not good for the development of the M. anisopliae fungus. According to (Solichah et al., 2022) that M. anisopliae can grow optimally at a temperature of 22-270C and humidity will experience a decrease in pathogenicity when below 86%. Environmental conditions are suitable, the M. anisopliae fungus will develop well. When environmental conditions are suitable, the M. anisopliae fungus will penetrate the insect's body through contact with the skin between the body segments (Susanti et al., 2012)). Conidia that enter the insect's body will reproduce through the process of forming hyphae in the epidermis and other tissues until they are filled with fungal mycelium.

3.2. Lethal Time 50 (LT50)

The dose of metankos application showed significant differences to Lethal Time 50 which occurred in the range of 3.75 days - 6.75 days. The application of metankos dose of 0.4 kg/plant (3.75 ± 0.95 days) tends to be the fastest and significantly different from the application of metankos 0 kg/plant (25.00 ± 0 days) but not significantly different from other doses, namely 0.3 kg/plant, 0.2 kg/plant and 0.1 kg/plant. The occurrence of unreal differences in LT50 is due to the observation at the beginning of death also gives an unreal effect. It is suspected that this occurs because of the low density of conidia of M. anisopliae fungus, which makes it unable to decompose the chitin and fat layers of the insect cuticle so that penetration and infection do not occur. (Yuningsih & Widyaningrum, 2014) stated that a high concentration with a larger number of conidia resulted in more conidia attached to the larval cuticle so that it infected larvae faster than a low concentration with a small number of conidia.

Table 2. Early mortality of *Oryctes rhinoceros* with Metankos application in oil palm nurseries

Metankos dosage	Lethal Time 50 (Days)
0.4 kg/plant	3.75 ± 0.95 a
0.3 kg/ plant	4.75 ± 0.95 a
0.2 kg/ plant	5.00 ± 0.81 a
0.1 kg/ plant	6.75 ± 1.70 a
0 kg/ plant	25.00 ± 0 b

Numbers followed by different lowercase letters are significantly different according to the DNMRT test at the 5% level. After being transformed with √y

The ability of M. anisopliae in controlling pests is not always the same, because M. anisopliae is host specific so that if the host is not suitable or less specific, the time to infect pests will be longer. According to Ginting et al. (2008), the time to infect target pests is influenced by the isolate used, the type of host and environmental conditions.

3.3. Daily Mortality

The addition of M. anisopliae doses to the diet of *Oryctes rhinoceros* larvae resulted in a notable increase in mortality. The highest mortality rate was observed at a dose of 0.4 kg/plant methancos, particularly during the period between days 5 and 16 (Figure 1). The observed increase in daily mortality can be attributed to the time required for the M. anisopliae fungus to infect its host. This is evidenced by the fact that the increase in mortality was only observed on days 7 to 16. The data on daily mortality are presented in Figure 3.

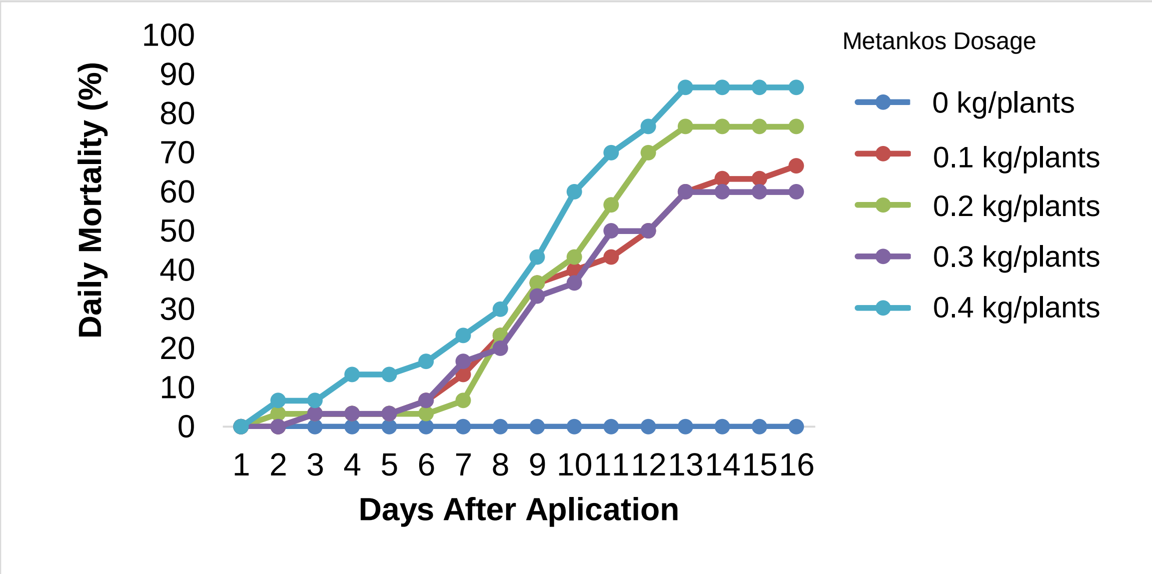

Figure 3. Daily mortality of *Oryctes rhinoceros* with metankos application in oil palm nurseries

The results of observations can be seen that daily mortality at a dose of metankos 0.4 kg/plant occurred on day 2 (hsa), a dose of metankos 0.3 kg/plant 3 (hsa), a dose of metankos 0.2 kg/plant on day 6 (hsa), a dose of metankos 0.1 kg/plant on day 7 (hsa) and at a dose of metankos 0 kg/plant no mortality occurred until the end of the study (Figure 1). This is thought to be because the higher the dose of metankos, the more conidia M. anisopliae will be, so it will be faster in killing *Oryctes rhinoceros*. According to (Permadi et al., 2019) conidia attached to insects take time to carry out infection through the cuticle, so it takes time to infect *Oryctes rhinoceros* larvae and mortality is also getting longer.

Figure 3 shows that the increase in mortality was highest at a dose of 0.4 kg/plant of metankos on days 2 to 16. This occurred because the dose given was the highest, so was the conidial density, so the infection power would be higher. According to Prayogo (2006), the addition of conidia density is one of the requirements to increase the effectiveness of entomopathogenic fungi.

The increase in daily mortality at a dose of methancos of 0.1 kg/plant to 0.4 kg/plant from day 6 to day 16 showed that increasing the concentration was able to increase the effectiveness of M. anisopliae in infecting *Oryctes rhinoceros*. This is supported by (Hasnah et al., 2012) who stated that the effectiveness of insect pathogenic fungi to control target pests is highly dependent on the density of the spores applied. Trisawa (2017) added that higher concentrations of M. anisopliae resulted in a greater number of fungal conidia entering the insect's body compared to less treatment.

3.4. Total Mortality

The application of a dose of metankos to control *Oryctes rhinoceros* pests in oil palm seedlings on peat significantly affects the total mortality of *Oryctes rhinoceros* in oil palm seedlings.

Total mortality of *Oryctes rhinoceros* with the application of metankos doses was significantly different with a range of 23.33% - 40%. Application of a dose of 0.4 kg/plant (40.00 ± 4.32%) was significantly different from the dose of 0.1 kg/plant (23.33 ± 3.09%) and 0 kg/plant (0%) but not significantly different from the dose of 0.3 kg/plant (36.00 ± 4.32%) and the dose of 0.2 kg/plant (33.33 ± 3.77%). This is thought to be due to the content of M. anisopliae in the compost that is able to kill *Oryctes rhinoceros* with 40% mortality. The low mortality of *Oryctes rhinoceros* in the nursery is thought to occur due to low humidity, which ranges from 56-78% so that M. anisopliae does not germinate well. The low mortality of *Oryctes rhinoceros* indicates that the application of M. anisopliae is still not capable as a bioinsecticide in controlling *Oryctes rhinoceros* pests in oil palm nurseries in peat soil. This follows the opinion of (Steinhaus, 1969) in (Hasyim, 2007) that fungi that can be categorized as bioinsecticides are fungi that successfully control insects with a mortality of 72.5% - 95%. This low result is also suspected to be due to the low concentration given so that it needs to be increased.

Table 3. Average total mortality of *Oryctes rhinoceros* with Metankos application in oil palm nursery

Metankos Dosage	Total mortality (%)
0.4 kg/plant	40.00 ± 4.32 a
0.3 kg/ plant	36.00 ± 4.32 a
0.2 kg/ plant	33.33 ± 3.77 a
0.1 kg/ plant	23.33 ± 3.09 b
0 kg/ plant	0 ± 0 c

Numbers followed by different lowercase letters are significantly different according to the DNMRT test at the 5% level. After being transformed with Arc sin√ y

The low mortality of *Oryctes rhinoceros* in oil palm nurseries is thought to occur due to humidity, temperature and climate that are less suitable for the growth of M.anisopliae fungi so that it is less effective in controlling *Oryctes rhinoceros* pests. According to Thaib et al 2013, the ideal temperature and pH for the propagation of M. anisopliae conidia are 25-30°C and pH 7. Based on the explanation (Solichah et al., 2022) that the infection and spread of M.anisopliae conidia are influenced by several factors, namely, wind, humidity and host density.

3.5. Height Increase of Oil Palm Seedlings

Figure 4 shows that the increase in the dose of methancos biocompost on the increase in plant height in oil palm plants has increased every month for 3 months of planting. This increase in height is thought to be due to the provision of compost fertilizer which is able to provide optimal nutrients for the growth and development of oil palm plants. Based on the research results of Fauzana et al. (2023a) that methancos contains high nutrients, namely pH 7.04, N 0.72%, P 0.82 mg/100 g, and K 4.32 mg/100 g. Thus, the provision of methancos can increase the growth of oil palm plants. According to (Wawan, 2017), an increase in the growth of oil palm seedlings accompanies an increase in the dose of fertilizer.

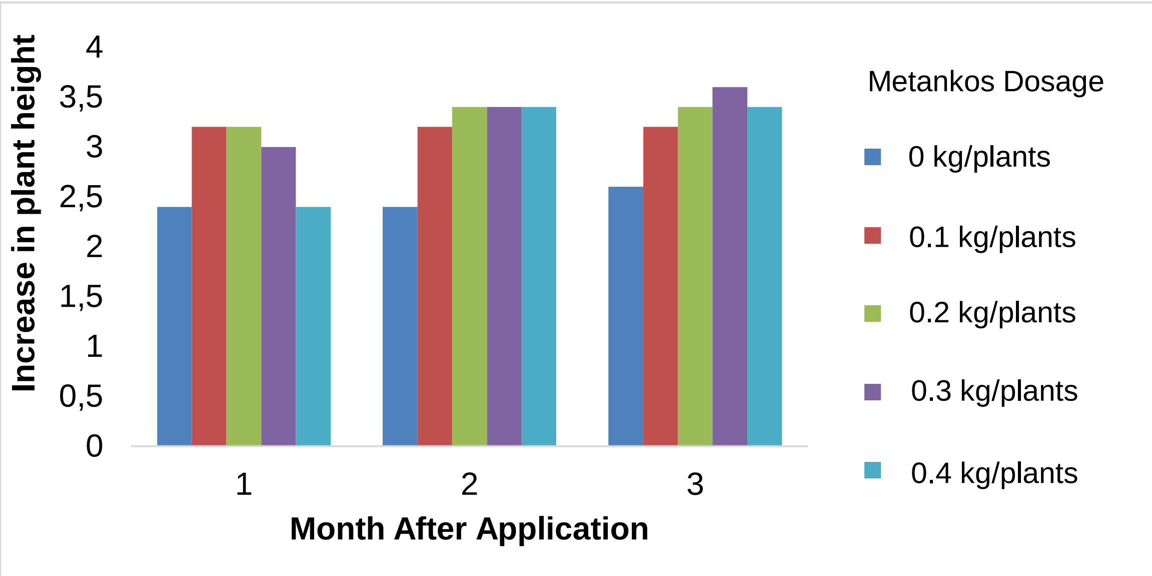

Figure 4. Increase in plant height with Metankos application in oil palm nurseries

The administration of methancos doses at 0 kg/plant, 0.1 kg/plant and 0.2 kg/plant experienced an increase that tended to be less than the better methancos doses of 0.3 kg/plant and 0.4 kg/plant. This is because the increase in plant height is closely related to the availability of nutrients, the provision of optimal nutrients will provide maximum growth effects on plants. Thus, the 0.3 and 0.4 kg/plant doses are thought to provide optimal nutrients.

3.6. Leaf Number Increase

The provision of methancos increased the number of leaves that occurred at all doses of methancos treatment (Figure 5). This is because providing methancos can improve soil nutrients as a growing medium for methancos plants, providing nutrients for oil palm seedlings. The results of the study by Leonardo et al. (2016) showed that the availability of N, P, and K nutrients had a significant effect on cell division, affecting the number of leaves produced by oil palm seedlings. The elements N, P, and K in the planting medium can stimulate cell division and expansion so young leaves can obtain their optimal shape more quickly.

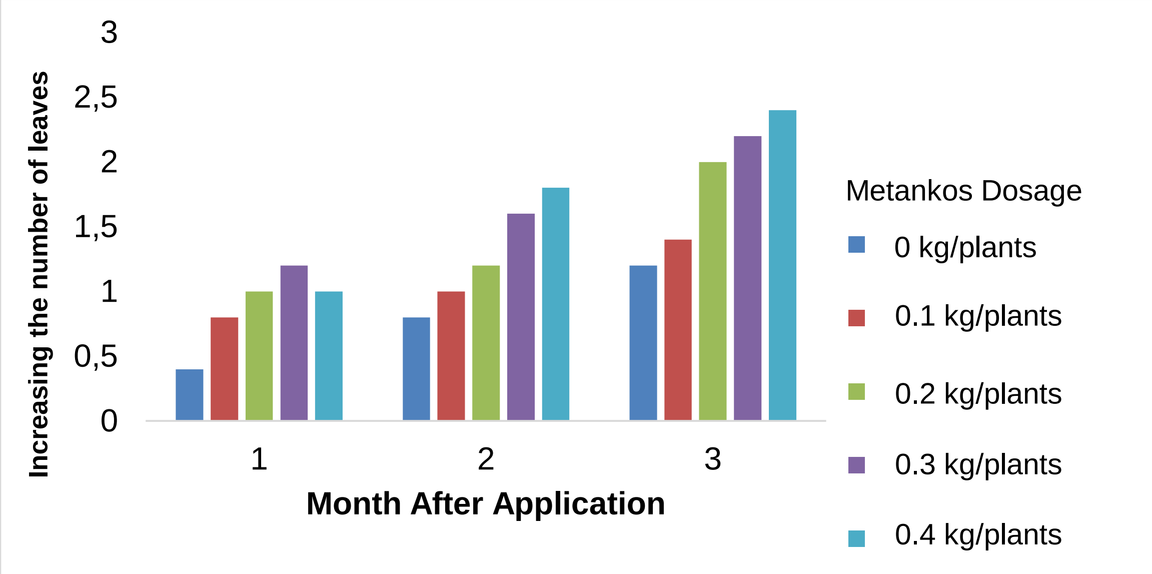

Figure 5. Increasing the number of leaves with the application of Metankos in oil palm nurseries

The availability of nutrients in the soil affects the process of leaf formation, especially nitrogen and phosphate. According to (Nurshanti, 2009) leaf growth will change rapidly and can accelerate the vegetative growth of plants because the absorption of N nutrients can increase the formation and growth of leaves in plants. The availability of N in sufficient quantities will facilitate plant metabolism and ultimately affect the growth of organs such as stems, leaves and roots to be good. The roots will absorb the nutrients needed by plants in vegetative growth so that the stems grow tall and affect the number of leaves.

3.7. Stem Diameter Increase

The increase in diameter at each additional dose of methancos increased every month of observation. This is suspected that the addition of methancos can provide good results for the increase in plant diameter. According to (Puspadewi et al., 2016), the planting medium given compost can provide good stem diameter development due to the availability of P and K nutrients.

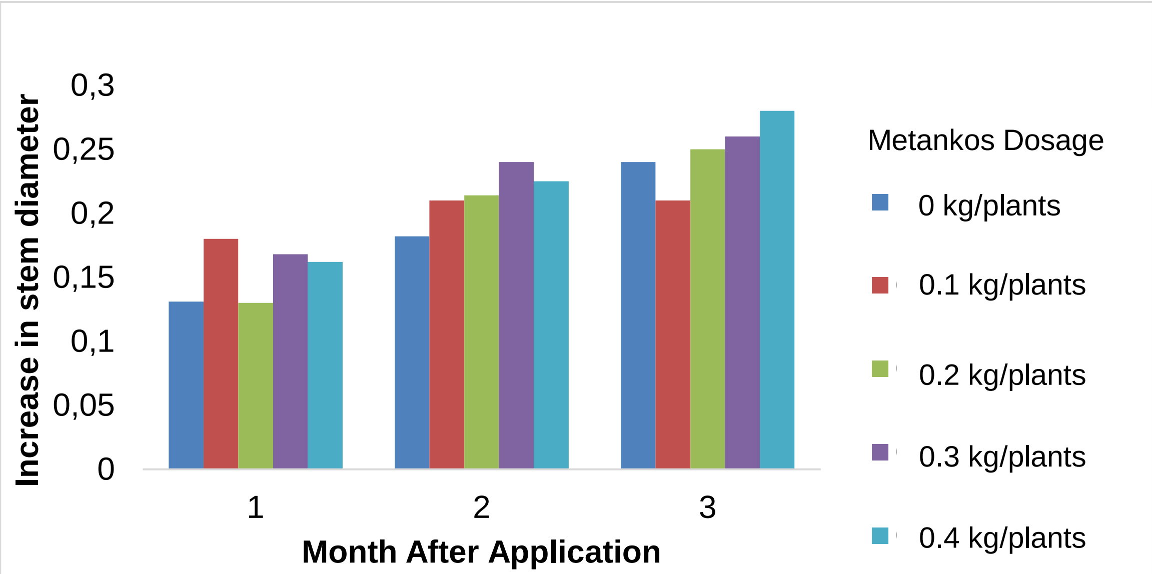

Figure 6. Increase in stem diameter with Metankos application in oil palm nurseries

The increase in stem diameter is observed to be more pronounced when a methane dose of 0.4 kg/plant is administered (Figure 6), which is in accordance with the observed increase in the number of leaves. It is hypothesized that this is due to the fact that a methacos dose of 0.4 kg/plant has been observed to facilitate superior vegetative growth outcomes in oil palm plants when compared to other methacos dose treatments. The findings of the study (Nurhadi et al., 2023) indicated that the acceleration of the growth of pre-nursery oil palm seedlings can be achieved through the administration of compost derived from empty oil palm bunches at a dosage of 30 g/polybag. It is hypothesized that the provision of optimal fertilizer will yield the most favourable outcomes. According to Yuniarti et al. (2020), the availability of elements needed by plants in sufficient conditions will facilitate the formation of proteins, enzymes, hormones and carbohydrates, thereby enabling rapid enlargement, elongation and cell division.

4. CONCLUSION

The study reaches the following conclusion:

1. The optimal dose of Methancos for the mortality of *Oryctes rhinoceros* larvae has been determined to be 0.4 g of Methancos/plant, resulting in 40% mortality, an initial death at 3.75 days, and a lethal time of 50 for 7.8 days, which does not reach the effective dose.
2. The optimal dose of methancos for the growth of oil palm seedlings is 0.4 kg/plant, as this has been found to result in the greatest increase in height, number of fronds and diameter.

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